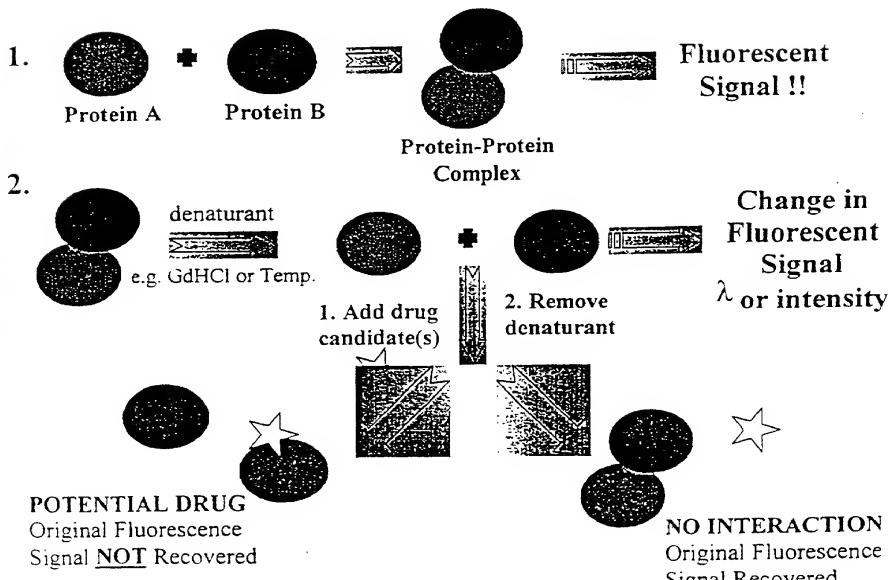


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## FIGURE 1

## Proposed Assay

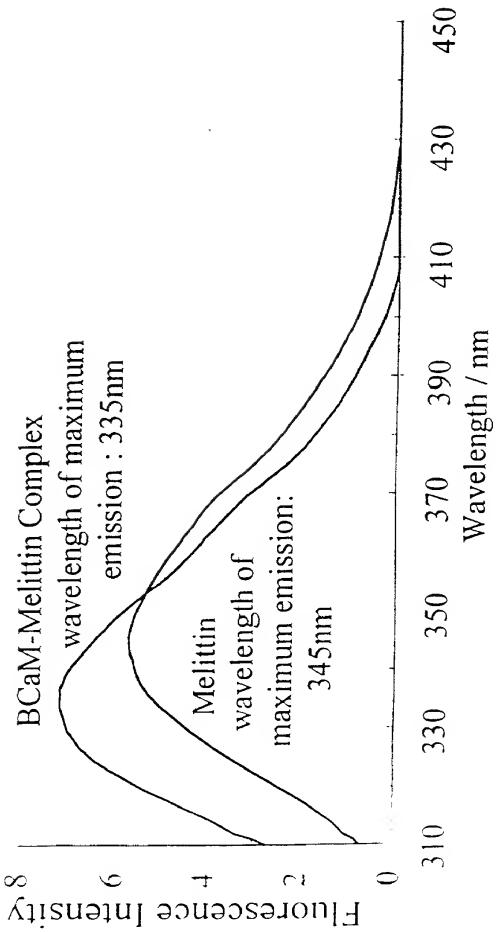


ENTRAP PROTEIN-PROTEIN COMPLEX IN A SOL-GEL  
DERIVED GLASS

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## FIGURE 2

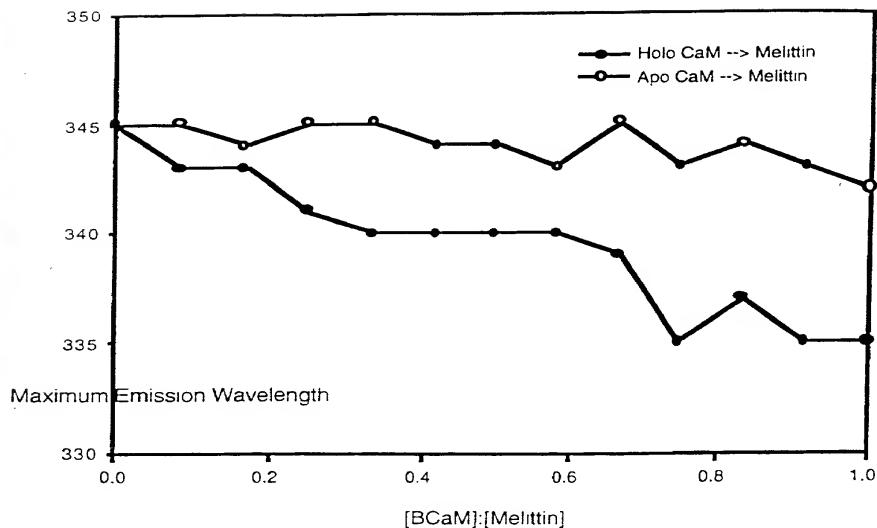
## Fluorescence Signal from BCaM-Melittin System



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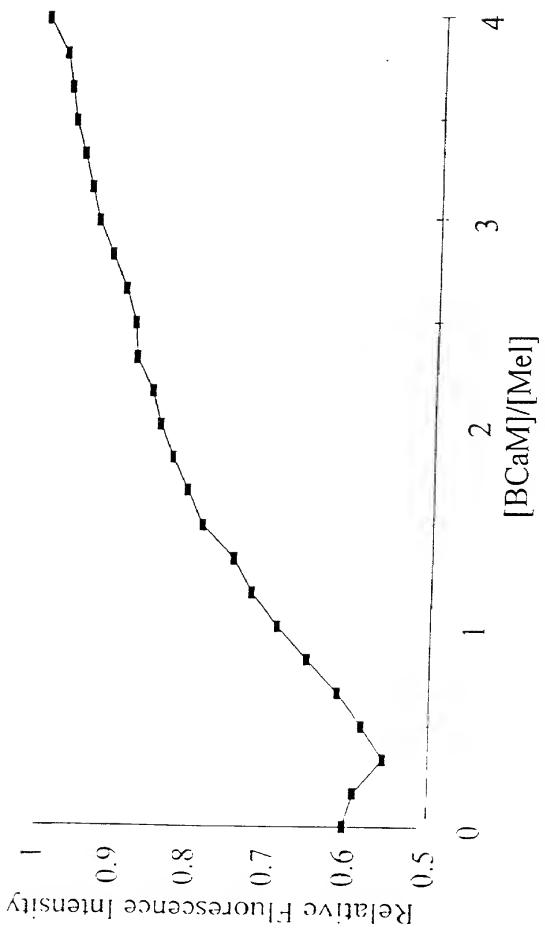
## FIGURES 3



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## FIGURE 4



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## FIGURE 5

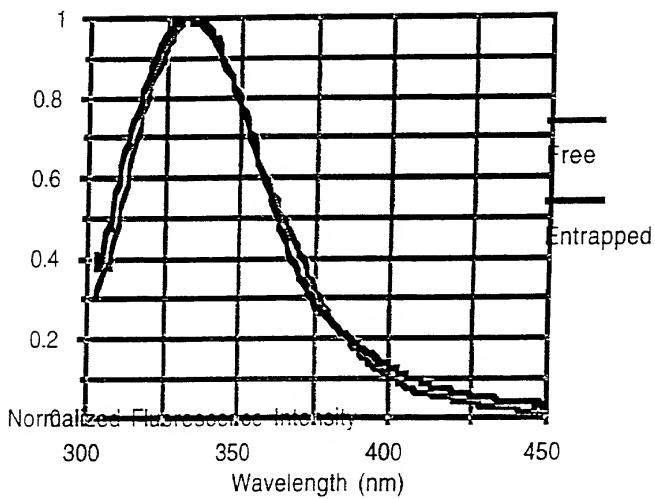
# The Sol-Gel Process

- 1)  $\text{Si(OEt)}_4 + \text{H}_2\text{O} + \text{H}^+ \longrightarrow \text{Si(OEt)}_{4-n}(\text{OH})_n + n \text{EtOH}$  *Hydrolysis*
- 2)  $2 \text{Si(OEt)}_{4-n}(\text{OH})_n \longrightarrow (\text{OH})_{n-1}(\text{OEt})_{4-n}\text{Si-O-Si(OEt)}_{4-n}(\text{OH})_n + \text{H}_2\text{O}$  *Condensation*
- 3)  $n \text{-Si-O-Si-} \longrightarrow [-\text{Si-O-Si-O-Si-O-}]_n + \text{H}_2\text{O}$  *Polycondensation*
- 4)  $n [-\text{Si-O-Si-O-Si-O-}]_n + \text{buffer + protein} \longrightarrow$  *Entrapped Protein* *Gelation*
- 5) Condensation and Polycondensation continue for days, shrinking gel *Ageing*

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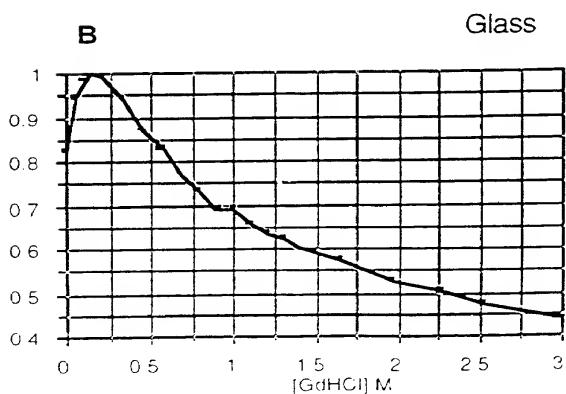
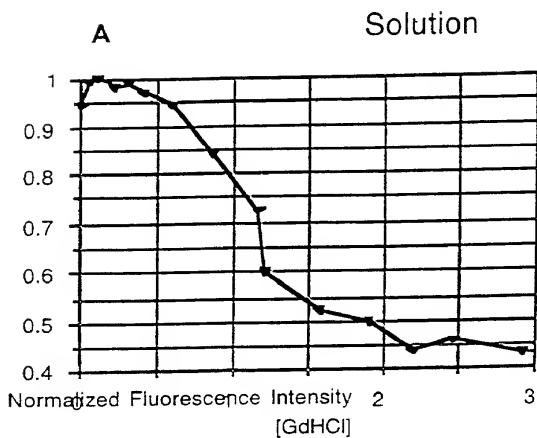
## FIGURE 6



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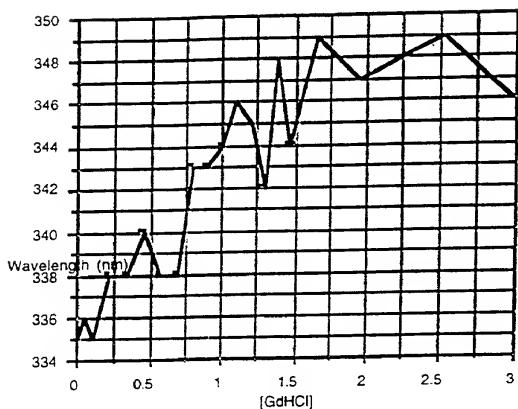
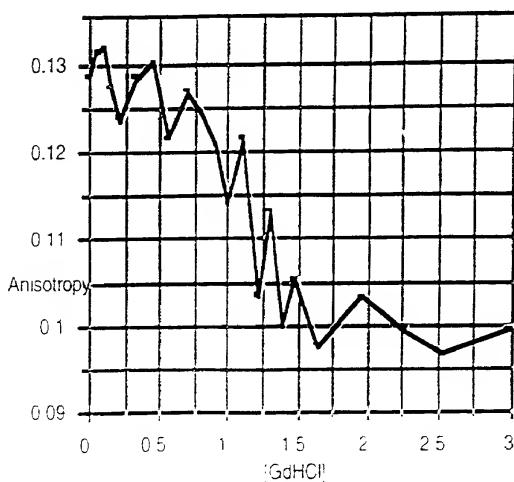
## FIGURE 7A-B



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## FIGURE 8A-B

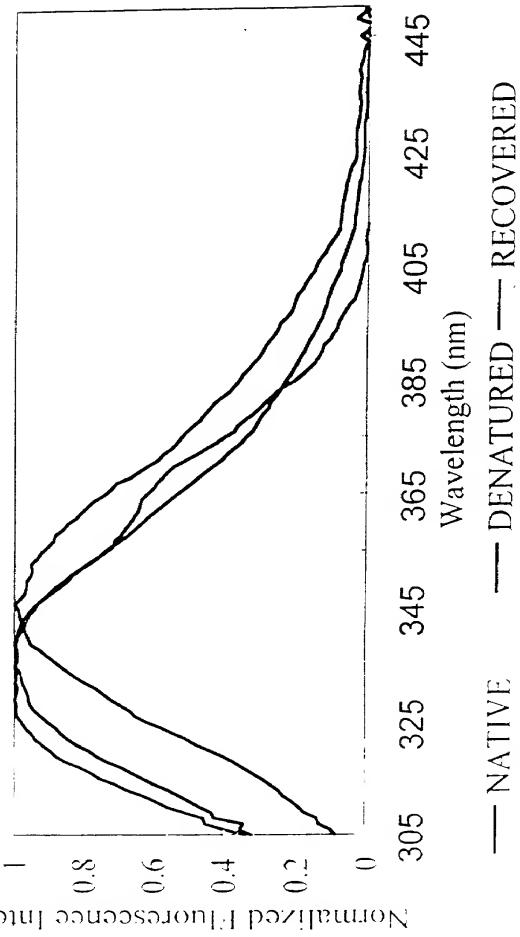
**A****B**

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FIGURE 9

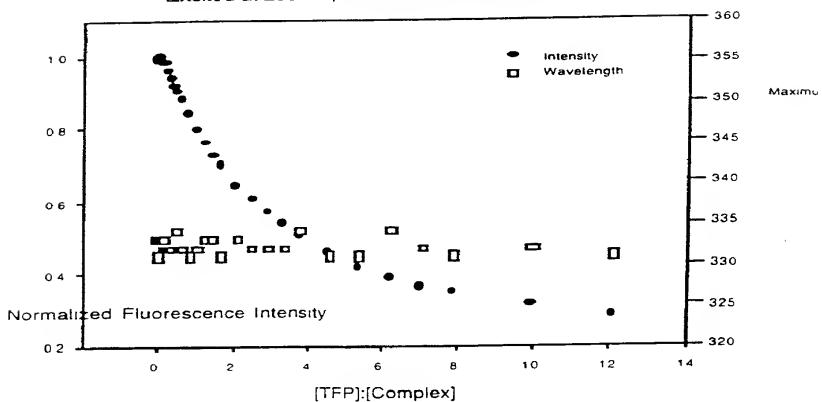
# Reversibility of Denaturation for an Entrapped Protein



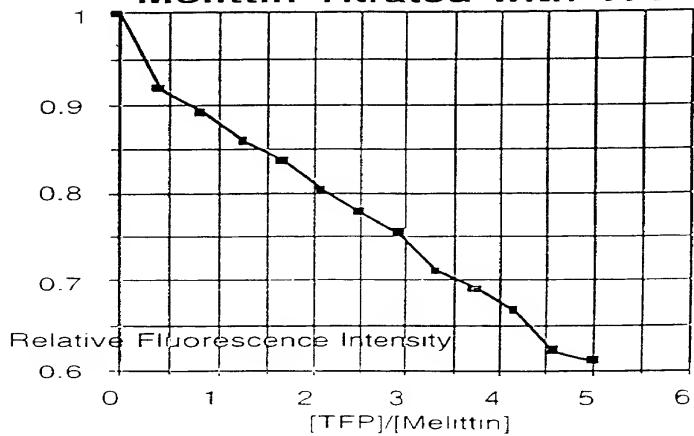
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## FIGURE 10A-B

**A TFP Added to BCaM-Melittin Complex**  
Excited at 295 nm, in the Presence of  $\text{Ca}^{2+}$



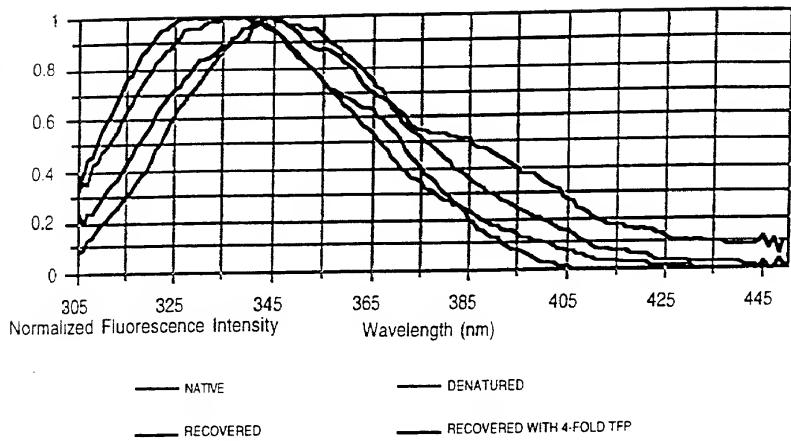
**B Melittin Titrated with TFP**



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## FIGURE 11



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FIGURE 12

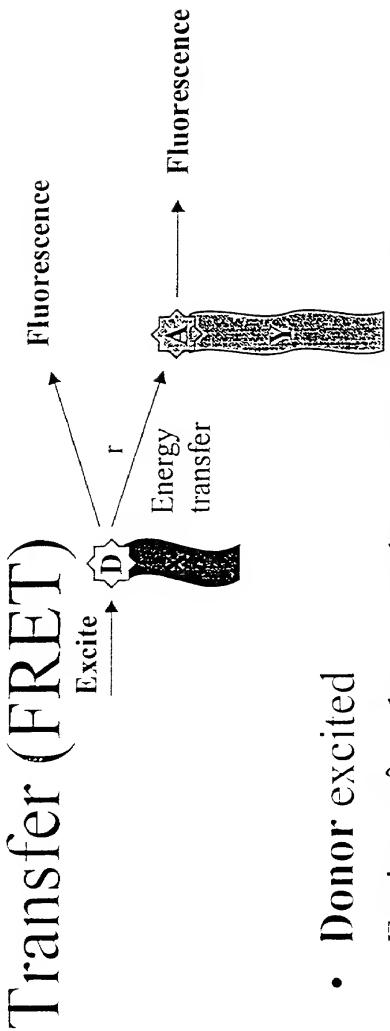
# Proof of Concept of the Assay

Solution	Anisotropy	Wavelength (nm)
Native	0.127	335
Denatured	0.099	346
Recovered	0.136	331
Denatured	0.115	346
Recovered with TFP	0.121	346

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FIGURE 13

# Fluorescence Resonance Energy Transfer (FRET)



- Donor excited
- Emits at  $\lambda_D$  that overlaps Acceptor absorbance
- Acceptor excited and emits at a longer  $\lambda_A$
- Efficiency of energy transfer dependent on distance between donor and acceptor
- Förster distance ( $R_0$ ) = 50% efficiency

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## FIGURE 14

- Donor attached to Protein A, Acceptor attached to Protein B
- Efficiency of energy transfer (E) related to distance (r) by;  
$$\frac{R_0^6}{R_0^6 + r^6}$$
- Use donor molecule which absorbs at longer  $\lambda$  to TFP :: avoid exciting TFP

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## FIGURE 15

Melittin

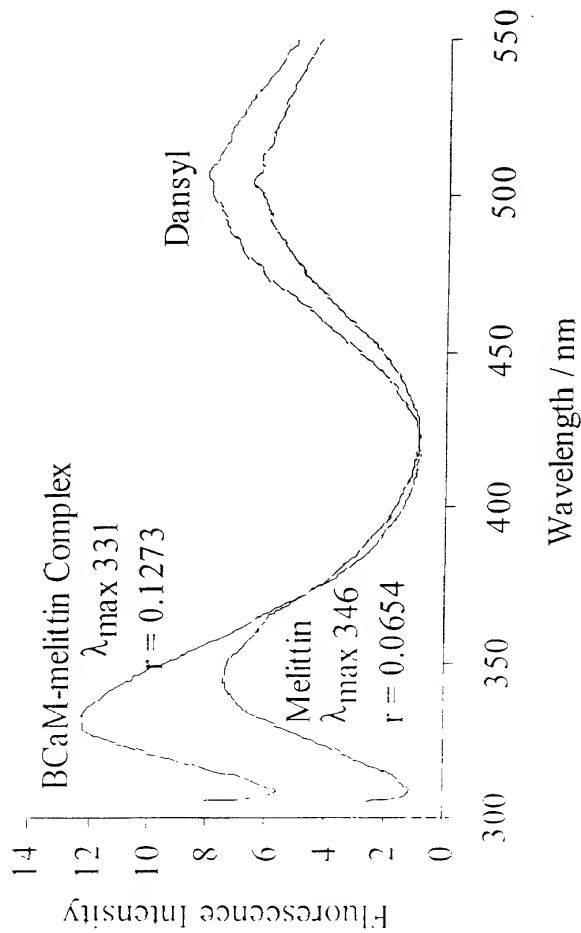
- Energy Donor
- Dansyl Chloride
- Absorbs @ 370nm
- Emits @ 500nm
- Energy Acceptor
- Fluorescein derivative
- Absorbs @ 494nm
- Emits @ 518nm

$$R_0 = 3.3 - 4.1 \text{ nm}$$

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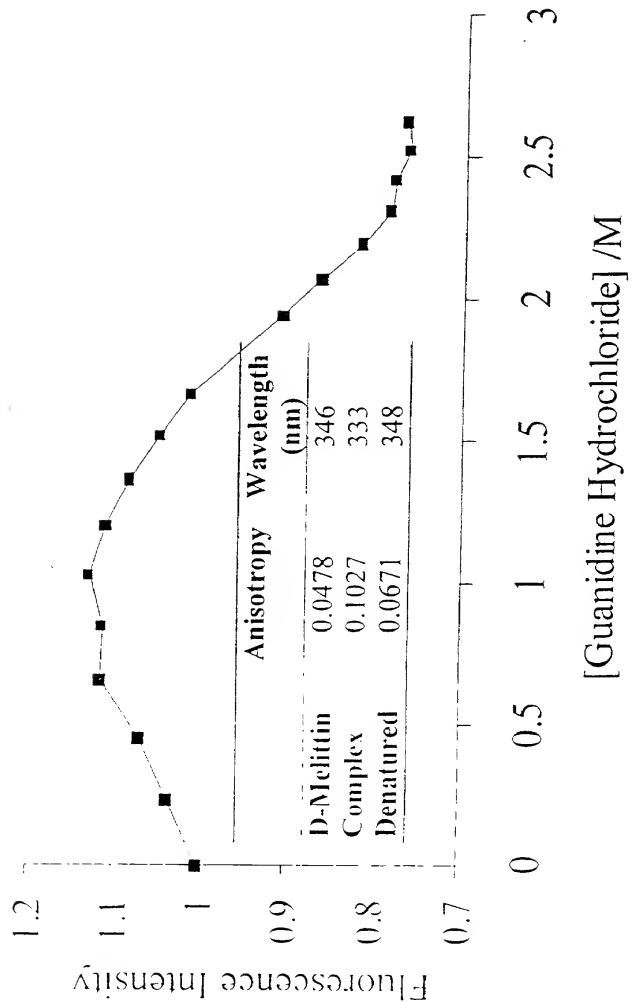
FIGURE 16



Dansyl doesn't interfere with PP interaction

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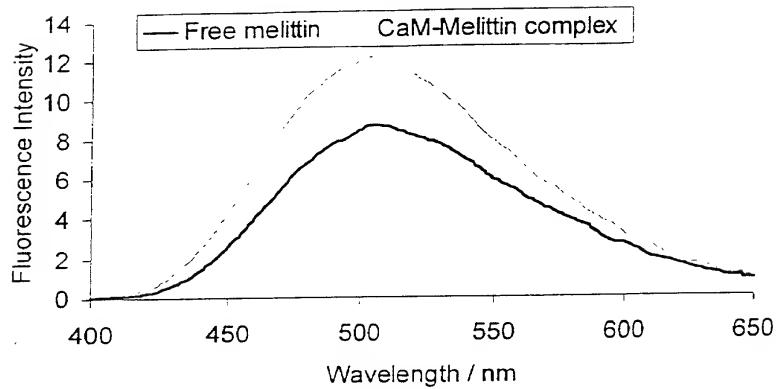
FIGURE 17



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## FIGURE 18

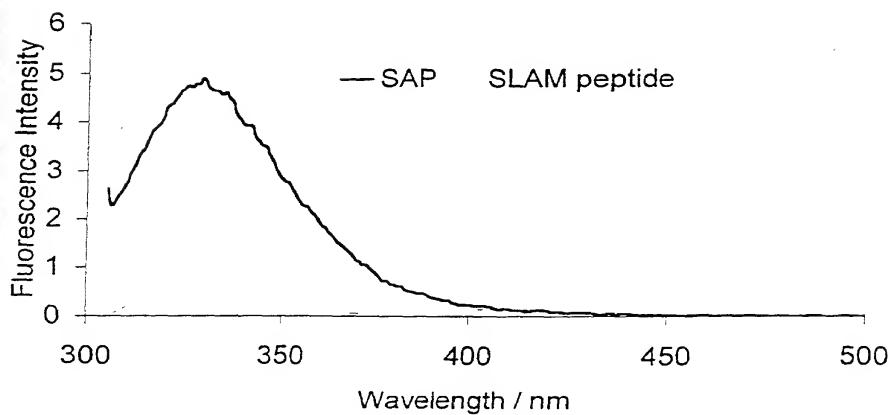


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## FIGURE 19

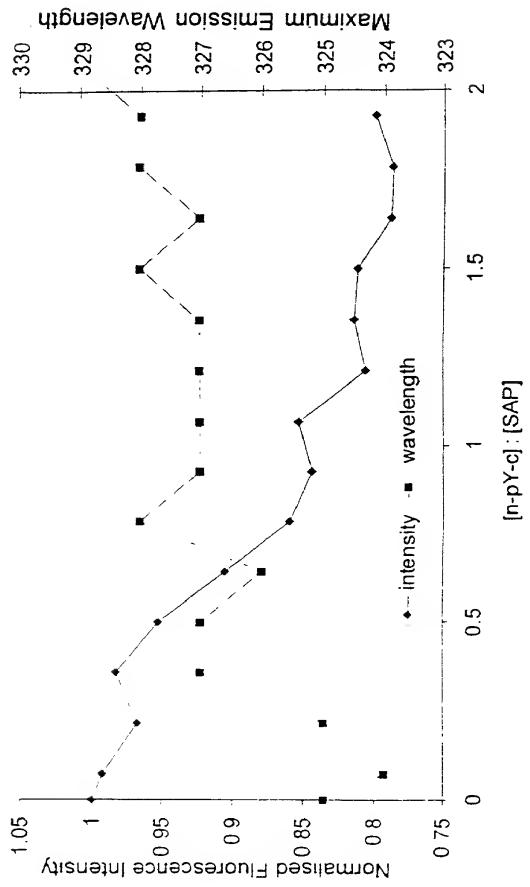
Fluorescence Spectra of SAP and Phosphorylated SLAM Peptide



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## FIGURE 20

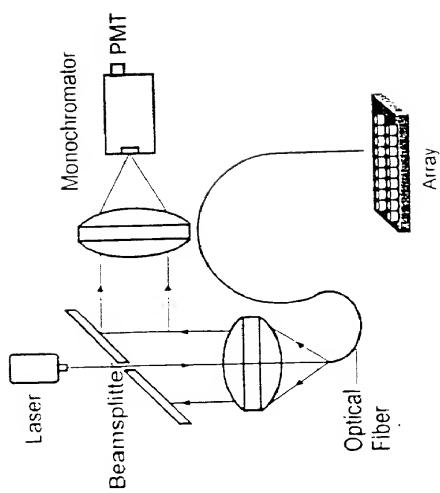
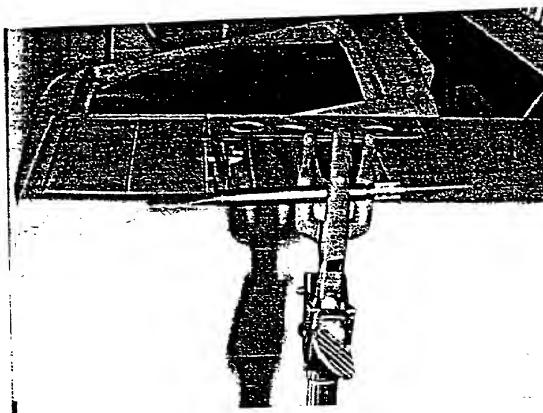
Titration of SAP with n-pY-c



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FIGURE 21



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FIGURE 22

